

Mechanisms of Voltage Sensing and Gating

3748-Pos Board B476

Generalized Interaction Energy Analysis of Intersubunit Linkage in Shaker Potassium Channels

Sandipan Chowdhury, Benjamin M. Haehnel, Baron Chanda.

Neuroscience, University of Wisconsin-Madison, Madison, WI, USA.

Voltage-dependent potassium channels are crucial for electrical excitability and cellular signaling; however, the molecular machinery that the channel employs, to relay the state of the voltage sensor to the pore, is not well understood. To gain insight into this voltage-transduction pathway, interacting networks need to be reliably mapped. Here we present a methodology to estimate the strength of site specific interactions called the Generalized Interaction-energy Analysis (or GIA). Our approach involves combining thermodynamic cycle analysis with information from the gating charge versus voltage curves of putative interactors. This methodology was benchmarked against well established kinetic models of Shaker potassium channels and BK channels using Monte Carlo like sampling. Our simulations show that GIA can provide free energy estimates in a self-consistent manner that will be useful to identify site-specific interactors that contribute to gating transitions. Implementing this approach on the Shaker potassium channel, we identify a cluster of highly conserved residues, located in the intracellular side of the channel pore, by the gate, that are energetically coupled. Specifically, it appears that tyrosine 485, on the S6 helix, is critical for maintaining the flexibility of an important hinge in the electromechanical coupling pathway.

3749-Pos Board B477

State-Dependent Lipid Interactions Couple the Conformations of the Voltage-Sensing and Pore-Gate Domains

Mark A. Zaydman¹, Marina Kasimova^{2,3}, Zachary Beller¹, Kelli Delaloye¹, Jingyi Shi¹, Jonathan R. Silva¹, Mounir Tarek², Jianmin Cui¹.

¹Biomedical Engineering, Washington University in St Louis, St Louis, MO, USA, ²Université de Lorraine, Nancy, France, ³Lomonosov Moscow State University, Moscow, Russian Federation.

Coupling between the voltage-sensing domain (VSD) and pore-gate domain (PGD) is required for the voltage-dependent gating of ion channels, but the molecular mechanisms of coupling are unclear. Previous studies have identified protein-protein interactions that are important for coupling, while structural and recent functional data demonstrate that membrane lipids also play a role. In a recent study of Kv7.1, we found that phosphatidylinositol 4,5-bisphosphate (PIP2) binding at the VSD-PD interface is required to couple the activated-state of the VSD to the open-state of the PD. We devised a method to directly measure PIP2 mediated coupling and an allosteric framework for describing such coupling. These advances provide new tools to investigate the mechanisms of VSD-PGD coupling. We also identified a putative PIP2 binding site and found that mutations of residues within this site reduce PIP2-mediated coupling. Paradoxically, a set of mutations near the PIP2 binding site increased the macroscopic current. Using a combined computational and experimental approach to study these gain of function mutations, we now identify a PIP2-interaction that is preferred by the resting-closed channel. Using the KCNE1 accessory subunit as an experimental tool, we are able to resolve the functional effects of this resting-closed state interaction. These results allow us to propose a novel mechanism for voltage-dependent gating in which repositioning of cofactor lipids at the VSD-PD represents a critical step in the transitions between resting-closed and activated-open states. This model can be used to explain the phenomena of cooperativity and concerted motion in voltage-gated channels.

3750-Pos Board B478

Probing Mechanisms through which Drugs Affect Voltage-Sensitive Gating

Zachary Beller, Mark Zaydman, Jianmin Cui.

Biomedical Engineering, Washington University, St. Louis, MO, USA.

Voltage-gated ion channels, which serve as the molecular basis for electrical signaling in excitable cells, represent a valuable target for drug therapy. However, there is a void in the mechanistic understanding of drug action on voltage-dependent gating. In our recent work on PIP2 regulation of Kv7.1, we developed a methodology to isolate the effect of PIP2 on three fundamental gating processes: voltage-sensing domain (VSD) activation, pore-gate domain (PGD) opening, and PGD-VSD coupling. Using Voltage Clamp Fluorometry (VCF) to track VSD movement simultaneously with ionic current to monitor PGD opening, we were able to gauge PIP2 effects on PGD opening and VSD activation. We detected coupling by performing VCF on locked-open channel mutants, allowing us to directly observe the impact of pore-opening on VSD activation through the VSD-PGD coupling. Here we demonstrate the ability of our methodology to provide mechanistic insights into drug effects using the potent Kv7.1 channel activator ML277 (Mattmann 2012). As reported previously, 10 μ M ML277 caused a maximal channel potentiation of around 4.5x initial current. We observed channel potentiation at 40 mV, at which point VSD activation is complete, indicating that ML277 acts on processes other than VSD activation. Using VCF, we see that

ML277 mildly right-shifts VSD activation in WT channels. This result contradicts pore-opener behavior, which would have left-shifted the fluorescence-voltage (FV) relationship. In contrast we see a dramatic left-shift in the FV relationship of the lock-open mutant, leading to the conclusion that ML277 strengthens the coupling process. Consistently, we saw a higher apparent affinity for ML277 in Kv380 mutants, which have been shown to exhibit increased PIP2 affinity. This study shows the utility of our methodology to analyze drug effects on voltage-sensitive gating.

3751-Pos Board B479

Single Molecule Fluorescence of an S4-Based Voltage Sensor

Jeremy S. Treger, Michael F. Priest, Tomoya Kubota, Ludivine Frezza, Francisco Bezanilla.

Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA.

Single-channel recordings revolutionized our understanding of voltage-gated ion channels by allowing observation of behaviors that are obscured by large ensemble averages. Yet despite its power, single-channel recordings only allow indirect inference about the motions of the voltage sensor since only transitions between "open" and "closed" states of the channel can be seen; most transitions between states remain hidden. For this reason, direct measurement of the motion of a single voltage sensor has long been a goal to understand the details of voltage sensing; unfortunately, at present the elementary charge transition is below experimental resolution. We report here observation of fluorescence from single voltage sensors conjugated to fluorescent proteins. These recordings respond to voltage and are able to recapitulate macroscopic recordings when averaged together. The protein we used is the "ArcLight" voltage sensor (Jin, L. et al. Neuron, 2012.), along with derivatives thereof. This sensor consists of the voltage sensing domain from Ci-VSP coupled to a GFP derivative, and it shows robust changes in fluorescence in response to voltage. Our recordings are taken from oocyte membranes using total internal reflection microscopy at a frame rate of 500 hertz and at a temperature of approximately 13 degrees Celsius. This combination of low temperature and fast acquisition allows detection of residencies of the protein at distinct fluorescence levels with stochastic movement between these levels being biased by voltage. Presumably these distinct fluorescence levels correspond to distinct states of the voltage sensor. The transitions between these states can be analyzed and modeled, producing a novel picture of how the voltage sensor moves and how these movements are influenced by membrane potential. Support: NIH GM030376.

3752-Pos Board B480

Effects of Decreased Hydrophobicity above R1 in S4-Based Voltage Sensors

João L. Carvalho-de-Souza, Jerome J. Lacroix, Francisco Bezanilla.

Biochem and Mol Biology, The University of Chicago, Chicago, IL, USA.

Membrane proteins containing S4-based voltage sensors domains (VSD) respond to changes in the membrane potential by transferring electrically charged side chains (gating charges), mainly located in the S4 segment, across the membrane electric field. The region just above the first charge (R1) is generally thought to control the VSD voltage-dependence. Here, we decreased residue hydrophobicity between N353 to L361 just above R1 in the Shaker Kv channels and found that for the two positions L358 and L361, less hydrophobic residues dramatically shifted the Charge vs. Voltage (Q-V) curve to more negative voltages, up to -132 mV. Screening L358 with several amino acids revealed a linear correlation between the side chain hydrophobicity and the Q-V curve midpoint. Interestingly, structural models of the VSD place the L358 and L361 side chains towards the hydrophobic core of the lipid bilayer in a resting state and towards the aqueous solvent in the active state, suggesting that less hydrophobic mutants may destabilize the resting state and/or stabilize the active state. To explore the origins of these phenotypes, we co-mutated some hydrophilic point mutations at position 358 or 361 together with the ILT mutation, which uncouples VSD movement from pore opening. Our results indicate that most double mutants shifts further the Q-V curves compared to the point mutations, but did not shift the G-V curves compared to the ILT mutant, suggesting that hydrophilic substitutions at positions 358 and 361 most likely destabilized the VSD resting state. Support: NIH-GM030376.

3753-Pos Board B481

Genetically-Encoded Fluorescent Voltage Sensors Capable of Resolving a 6mV Depolarization

Arong Jung.

Center for Functional Connectomics, KIST, Korea university, Seoul, Korea, Republic of.

Genetically encoded fluorescent sensors of membrane potentials utilize the voltage sensing domain of the voltage-sensing phosphatase (VSPs) gene family. Probes based on the *Danio Rerio* voltage-sensing domain exhibit fast optical responses of around 5 msec. Here we developed several potential optical sensors by mutating the voltage-sensing domain which consists of four transmembrane segments (S1-S4). Mutations to S4 changed the voltage response resulting in a